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AUTHOR(S):

栗田, 和紀

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Phylogeography and population genetics of the Ryukyu five-lined skink *Plestiodon marginatus*

Kazuki KURITA

Introduction

The Ryukyu five-lined skink, *Plestiodon marginatus*, is widely distributed in the Ryukyus (the Okinawa, Amami, and Tokara Groups), and inhabits in most islands within its range including even tiny islets. Several studies attempted to elucidate the genetic variation of this species, but the comprehensive picture of population-level relationships and the fine scale genetic structure have remained to be clarified. In this thesis, I studied the biogeography and population structure of the Ryukyu five-lined skink based on its genetic variation, and revised its taxonomy.

Chapter 1. Divergence and long-distance overseas dispersals of *Plestiodon marginatus* as revealed by phylogeographic analyses

I investigated the inter- and intraspecific relationships of *P. marginatus* to assess its historical biogeography. Especially, the aims are directed to reveal the origin, the timing, and the route of the colonization to three volcanic islands in the northern Tokara Group of the northern Ryukyus, Kuchinoshima, Nakanoshima, and Suwanosejima Islands. I conducted phylogenetic analyses and divergence time estimation using a partial sequence of the mitochondrial cytochrome *b* gene for 76 individuals of *P. marginatus* collected from across its whole range (26 localities of 23 islands in the northern and central Ryukyus), and *P. stimpsonii* (three islands from Yaeyama Group of the southern Ryukyus) and *P. elegans* (from Taiwan). The results showed that there

were three major clades (A, B, and C). Clades A and B consisted of *P. marginatus* excluding the Kuchinoshima population, and Clade C consisted of the Kuchinoshima population, *P. stimpsonii*, and *P. elegans*. These clades were estimated to have diverged during the Late Miocene to the Late Pliocene. These diversification dates were extremely older than that estimated in the previous studies. Among the three island populations of the northern Tokara Group, the Kuchinoshima population was shown to be a sister group of *P. stimpsonii*. The two other populations from Nakanoshima and Suwanosejima Islands were closely related to *P. marginatus*, the populations of the northern part of the Okinawa Group and the Kodakarajima population in the southern Tokara Group, respectively. These populations were estimated to have diverged from their respective relatives in the Early, Middle, and Late Pleistocene, respectively. These findings suggest that they colonized these islands by independent overseas dispersals of approximately 50–850 km via the Kuroshio Current.

The genetic distance between two subspecies of *P. marginatus* (Clades A and B) is comparable to the interspecific distances between congeneric species, and therefore I concluded that two subspecies should be regarded as distinct species.

Chapter 2. Taxonomic status of *Plestiodon* population from Kuchinoshima Island and its characteristics

I examined nuclear DNA and morphological variation of *Plestiodon* skinks from the Ryukyus to clarify the taxonomic status of the Kuchinoshima population. Nuclear DNA data supported the sister relationship between the Kuchinoshima population and *P. stimpsonii* as estimated by mitochondrial DNA phylogeny. The Kuchinoshima population was also divergent genetically in both mitochondrial and nuclear DNA markers, and morphologically in having unique scutelation and body coloration from other *Plestiodon* congeners in the Ryukyus.

Therefore, I regarded it as a distinct species.

Chapter 3. Population maintenance mechanism of island populations of *Plestiodon marginatus*

I explored the population maintenance mechanism of insular populations of *P. marginatus*. I examined the genetic variation of a partial sequence of the mitochondrial cytochrome *b* gene (n = 67) and ten microsatellite loci (n = 235) for 21 populations from 11 land-bridge islands that were connected during the latest glacial age, and four isolated islands. The results showed that moderate to high levels of genetic differentiations, existence of a number of private alleles/haplotypes in most islands, little contemporary migration, a positive correlation between genetic variability and island area, and a negative correlation between relatedness among individuals and island area. These evidences suggest that most island populations are maintained as isolated from each other under a strong effect of independent genetic drift as opposed to gene flow. Isolation-by-distance effect was demonstrated and it became more prominent when the four isolated islands were excluded, suggesting that the pattern is a remnant of the land-bridge age. In a few island populations, however, the possibility of occasional overwater dispersals was partially supported and therefore could not be ruled out.